

Claims

1- A method for selecting or preparing cells comprising at least one metabolic pathway
 5 or metabolic pathway family enabling the transformation of one or more substrate(s)
 {Ai} into a desired product {B}, comprising the following steps :

- a) providing a population of host cells (Ai- ; B-) incapable of metabolising said
 substrate or substrates {Ai} and said product {B} ;
- 10 b) transforming said population of host cells with a library of sequences of
 nucleic acid ;
- c) testing in parallel said population of transformed host cells on minimum
 media containing either one of the substrates {Ai}, or said product {B} as the
 only source of an element essential to growth; and,
- 15 d) selecting said host cell(s) capable of growth on a minimum medium
 containing one of the substrates {Ai} and on a minimum medium containing said
 product {B} (Ai+ ; B+).

2- The method according to Claim 1, comprising, before step c), a step consisting of
 testing said population of transformed host cells on a minimum medium containing the
 20 substrate(s) {Ai} and said product {B} as the only source of an element essential to
 growth and selecting said host cell(s) capable of growth on said minimum medium
 containing the substrate(s) {Ai} and said product {B}; said selected host cell(s) then
 being subjected to step c) and the subsequent steps.

25 3- The method according to Claim 1 or 2, comprising, after step d), the following steps :
 e) implementing *in vitro* mutagenesis of the molecule of nucleic acid isolated
 from said transformed host cell(s) (Ai+ ; B+) in step d) ;
 f) re-transforming the population of host cells (Ai- ; B-) described in step a) with
 the population of nucleic acids mutated *in vitro* in step e) and testing the host
 30 cell(s) thus transformed on minimum media containing either one of the
 substrate(s) {Ai}, or said product {B} as the only source of an element essential
 to growth; and,

g) selecting said transformed host cell(s) incapable of growth on a minimum medium containing one of the substrate(s) {Ai} and capable of growth on a minimum medium containing said product {B} (Ai- ; B+), and optionally isolating the mutated molecule of nucleic acid.

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4- The method according to Claim 3, comprising the characterisation of the gene or genes encoding the enzyme or enzymes involved in the conversion of the substrate {Ai} into product {B} in said host cell(s) (Ai- ; B+) selected in step g).

10 5- The method according to Claim 3, comprising, after step f), in parallel to step g):

h) selecting said transformed host cell(s) which has (have) become incapable of growth on a minimum medium containing one of the substrate {Ai} and on a minimum medium containing said product {B} (Ai- ; B-) ;

15 i) implementing a quantitative analysis of the accumulation of the product {B} of said transformed host cells(s) (Ai- ; B-) on a rich medium supplemented by {Ai} ; and

j) selecting said transformed host cell(s) (Ai- ; B-) accumulating the product {B} on a rich medium and optionally isolating in parallel the mutated molecule of nucleic acid introduced during the transformation of step f).

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6- The method according to Claim 5, comprising the characterisation of the gene or genes encoding the enzyme or enzymes involved in the conversion of the substrate {Ai} into product {B} in said host cell(s) (Ai- ; B-) selected in step j).

25 7- The method according to any of the preceding claims, comprising, after step c), in parallel to step d) and the subsequent steps, the following steps :

k) selecting said transformed host cell(s), incapable of growth on a minimum medium containing one of the substrates {Ai} and capable of growth on a minimum medium containing said product {B}, called receiving cell(s) (Ai- ; B+) ;

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l) transforming said receiving cell(s) (Ai- ; B+) with a library of sequences of nucleic acid;

m) testing in parallel said transformed receiving cell(s) (Ai- ; B+) on a minimum medium containing one of the substrate(s) {Ai} ; and

n) selecting said transformed receiving cell(s) capable of growth on a minimum medium containing one of the substrates {Ai} ; and

5 o) characterising the gene or genes encoding the enzyme or enzymes involved in the conversion of the substrate {Ai} into product {B} in said transformed receiving cell(s) (Ai+ ; B+) selected in step n).

8- The method according to Claim 7, comprising, before step m), testing said host
10 cell(s) (Ai- ; B+) transformed on a minimum medium containing several substrates {Ai} as the only source of an element essential to growth and selecting said host cell(s) capable of growth on said minimum medium containing several substrates {Ai}; said selected host cell(s) then being subjected to step m) and the subsequent steps.

15 9- The method according to Claim 7 or 8, wherein:

- between steps k) and l), said host cell(s) (Ai- ; B+) is/are modified by replacing the first selection marker present in the vector containing the sequence of nucleic acid introduced in step b) with a new selection marker ;

- said library of sequences of nucleic acid from step l) includes a selection marker
20 different to that carried by said host cell(s) (Ai-B+)

- the method further includes the following steps :

kk) the extraction and purification of the vectors contained in said host cell(s) selected in step k) ;

25 kkk) the *in vitro* mutagenesis of said vector purified in step kk), advantageously by transposition with a transposable element carrying a functional resistance to an antibiotic different to that previously existing on this vector.

kkkk) the transformation of said host cell(s) (Ai- ;B-) incapable of metabolising said substrate(s) {Ai} and said product {B} by the mutated nucleic acids obtained in the previous step ;

30 kkkkk) the selection of transformed host cells containing just said second selection marker ; these transformed cells, of phenotype (Ai-B+), called receiving cells, are then the object of the transformation described in step l).

10- The method according to any of the preceding claims, wherein said host cells are eukaryotic or prokaryotic cells.

11- The method according to Claim 10, wherein said host cells are :

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- cultivable under standard conditions known by the man skilled in the art,
 - transformable, and
 - capable of stably maintaining the transforming exogenous DNA.

12- The method according to Claim 10 or 11, wherein said host cells are bacteria.

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13- The method according to any of the preceding claims, wherein said library of sequences of nucleic acid is a metagenomic library.

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14- The method according to any of Claims 1 to 12, wherein said library of nucleic acid sequences originates from cultivatable prokaryotic or eukaryotic organisms.

15- The method according to any of Claims 1 to 12, wherein said library of nucleic acid sequences originates from non-cultivatable prokaryotic or eukaryotic organisms.

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16- Use of a host cell selected in step g) of Claim 3, or in step j) of Claim 5 in a process for preparing the product {B} from the substrate {Ai}.

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17- Use of a host cell transformed with the gene or genes encoding the enzyme or enzymes involved in the conversion of the substrate {Ai} into product {B} characterised according to Claim 4 or 6 in a process for preparing the product {B} from the substrate {Ai}.

18- A method for selecting or preparing a host cell (Ai- ; B-) incapable of metabolising said substrate(s) {Ai} and said product {B} comprising the following steps :

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- testing a population of host cells, cultivatable under standard laboratory conditions and under industrial production conditions, transformable, and capable of stably maintaining the transforming exogenous DNA, on a minimum

medium containing the substrate(s) {Ai} and said product {B} as the only source of an element essential to growth; and,

- selecting the host cell(s) incapable of growth on said minimum medium containing the substrate(s) {Ai} and said product {B}.